Topographically enhanced cell culture systems to induce and monitor mechanobiology

Chapter 9

Valorization

Introducing the TopoWellPlate in cell culture laboratories

Introduction

In this chapter, we discuss the valorization opportunities for the results obtained in this thesis. The definition of valorization, as provided by the National Valorization Committee (2011:8), stated that: "With knowledge valorization one creates value from knowledge. For this, knowledge needs to be suitable and/or available for social and/or economic use"[1].

Valorization has been an often encountered topic throughout the course of my PhD-trajectory. Both the laboratory of Cell Biology Inspired Tissue Engineering (cBITE) and Materiomics BV use the TopoChip platform, which repeatedly highlighted our differences in priorities. Materiomics aims to commercialize the TopoChip platform via patented surface topographies which can improve the interaction between implanted materials and the human body. In contrast, within cBITE we focus on unravelling the underlying mechanisms of mechanotransduction by which surface topography enhanced biomaterials control cell behavior. This results in a great conflict of interest. Where cBITE wants to share its scientific findings as broad as possible, Materiomics needs to keep all its findings secret till a patent has been filed. Moreover, cBITE's output model consists of publications in scientific journals, whereas Materiomics' output model involves filing patents and the major issue is the difference in lag time and the impact of publishing on novelty claims. A scientist who hinted at a certain molecular mechanism of surface topography-induced cell signaling in the discussion section of a minor article can still publish a Nature paper on that topic years later. In contrast, a correlation on topography design and functionality presented at the obscurest of conferences can block the protection of a block buster application of that correlation years later. Therefore, Materiomics is more cautious with disclosing information in the form of patents or scientific publications than cBITE, resulting in a longer lag time. Nevertheless, Materiomics and cBITE can benefit from one another, in terms of methods development, data analysis and obtained results. However, such a relationship should follow certain rules in order to protect the intellectual property of the TopoChip platform. As a consequence, obtained results were – without exception – handled as confidential. Using images that revealed material details and its accompanying data were not to be presented, and some of the chapters in this thesis could only be published after the patents were filed. To supervise this confidentiality, a strict procedure was followed for all output coming from cBITE before it was published in any way. This included revision by the cBITE chair first, followed by strategic considerations about the presented findings by Materiomics' business development team and CEO. Altogether, it is evident that valorization and scientific research can go hand-in-hand but one needs to know the rules that apply to both become successful.

Societal impact of this thesis

Scientists try to gain knowledge and spread this knowledge in the public domain. Between researchers, scientific communication occurs mainly via publications in peer-reviewed journals or at conferences and symposia. However, in order to create societal awareness around research topics and findings, there is a need to reach out via different routes as well.

The work presented in this thesis was largely financed by European Union, as the STELLAR project (Grant Agreement No. 305436) under the Seventh Framework Program (FP7/2007-2013). Within the STELLAR consortium – coordinated by the Nephrology department of the Leiden University Medical Centre (LUMC) – we strived for kidney function regeneration for patients suffering from chronic kidney diseases. In order to create societal awareness, significant energy was put in the communication of our work towards the public. Besides a regular website (http://www.stellarproject.eu/), the consortium invested in their visibility using social media (https://nl-nl.facebook.com/StellarStemCellsInKidneyDisease/). Via these routes, a follower base of hundreds of interested people (scientists and non-scientists) was created (October 2017), which led to many interesting discussions and collaborations in science as well as in art. Furthermore, the head of the STELLAR consortium (prof. Rabelink, LUMC Nephrology) appeared in a public television show on January 20 2015, (Tijd voor MAX) to elaborate on the latest progress made in kidney regeneration research (https://www.omroepmax.nl/pers/persberichten/september/tijd-voor-max-goede-doelenspecial-de-nierstichting/). This resulted in an information flow from our international research consortium towards the public domain, where many patients and their surroundings were reached, as well as our peers. The content of chapter 6, in which we identified conditions to optimise kidney stromal cell biology, particularly fitted this outreach strategy.

Chapters 3 and 4 comprise a more fundamental type of research, and the societal impact of this work seems to be less prominent. These chapters are in a sense more focussed on gaining knowledge. This knowledge can be useful in future research to elucidate the mechanism of action in biomaterials-controlled cell behaviour. Subsequently, we can design biomaterials rationally to perform specific tasks at the site of implantation. This could potentially result in additional positive effects of the biomaterials on their direct environment, and at the same time, diminished influence of negative side effects. Although the knowledge gained in chapter 3 and 4 does not directly create societal impact at this moment, it could be the basis of future discoveries with more impact since it contributes to the development of a platform technology.

The TopoWellPlate platform developed in chapter 5 did already progress along the Technology Readiness Level (TRL) scale (table 1). To assess technological development, this guideline was introduced by the NASA in 1974. More recently, the guidelines were adapted by the European Union, where it is now part of the Horizon 2020 guidelines[2]. The TRL-scale reaches from basic technology research (TRL1) towards full testing of an operation (TRL9).

The TopoWellPlate is a platform with potential to be further developed into an industrial product. Besides the prototype fabrication and proof of principle as presented in chapter 5 (TRL1/2), chapter 6 adds a more mature feasibility study for the TopoWellPlate concept (TRL2/3). However, full maturation (TRL9) of this product is needed in order to obtain its potential economic value.

Table 1: Technology Readiness Level definitions as published by the European Union. (Horizon2020 – Work Programme 2014-2015, General Annexes G)

Technology Readiness Level	Description
TRL 1	Basic principles observed
TRL 2	Technology concept formulated
TRL 3	Experimental proof of concept
TRL 4	Technology validated in lab
TRL 5	Technology validated in relevant environment
TRL 6	Technology demonstrated in relevant environment
TRL 7	System prototype demonstration in operational environment
TRL 8	System complete and qualified
TRL 9	Actual system proven in operational environment

Opportunities for the TopoWellPlate

In previous work using the TopoChip platform, we showed the beneficial properties of surface topographies over unpatterned tissue culture plastics for multiple biological models. For example, a prolonged hepatocyte viability *in vitro*[3], xeno-free IPSC stemness maintenance and self-renewal[4], and morphology specific secretion profiles of kidney-derived stromal cells (chapter 6 of this thesis).

Hepatocytes are the major cell type in the liver and account for more than 70% of its total tissue weight. Due to the detoxification activity of the liver, hepatocytes are used in drug development screening experiments by pharmaceutical companies. Currently, the golden standard for culturing hepatocytes only allows for an 8 day period to perform experiments, after which cell viability significantly reduces and cells detach. However, Materiomics identified surface topographies which increased the lifespan of hepatocytes for up to 30 days. Providing pharmaceutical companies with TopoWellPlates which are optimized for hepatocyte culture might therefore be of great interest to both parties. To be able to provide such a platform to these companies, Materiomics needs to further progress along the TRL-ladder with the TopoWellPlate platform. In order to do so, they assessed the current production method of the TopoWellPlate prototype and started exploring alternative production methods, which will be discussed in the next paragraph.

The need for the TopoWellPlate in drug development is based on the poor translation between the studied disease model and the current *in vitro* experimental set-up. Cells which are isolated from the human body experience a dramatic change in microenvironment when cultured on tissue culture plastic. The cells' new environment causes changes in cellular

phenotype, and alters functionality and potency. In drug development, testing of novel compounds is preferably performed on human primary cells in order to create a biological model as close as possible to the *in vivo* situation. However, due to afore mentioned reasons, the *in vitro* models are often far from ideal. Furthermore, the use of primary cells can be accompanied by more difficulties, as seen by the decrease in viability in the hepatocyte example. As an alternative, drug screening is typically performed on cell lines. And after the screening phase, the selected drugs need to be tested in animal models. Using topographies – which create an optimized culture environment for human primary cells – might help to overcome the need for animal studies, since the *in vitro* work takes up less time, and is performed in a more relevant biological system. Pharmaceutical companies are interested in the TopoWellPlate system since animal studies take up a large fraction of the total drug development budget (annually between 100 million and 1 billion)[5]. Reduction of these costs using TopoWellPlate technology might be very attractive in the pharmaceutical industry, where a total of approxamitely 55 billion dollars is spend on research and development for drug development[6].

Besides creating a product for the pharmaceutical industry, the TopoWellPlate can be also used in many other research facilities around the world. Of note, compared to the research and development budget in the pharma-industry, there is significantly less money spent in the academic world for the possible application of TopoWellPlate technology. As mentioned before, the TopoWellPlate can be used as a cell culture system to study models known to contain a mechanobiological component. More-and-more models are described to have a mechanosensitive mechanism. Let's highlight one example from this wealth of mechanoresponsive biological models, e.g. induced pluripotent stem cell (IPSC) culture. Since the introduction of IPSCs in 2006, many laboratories around the world used the Yamanaka factors to reprogram adult cells into pluripotent cells. Since IPSCs can potentially become every cell type of the human body and also divide infinitely, it is seen as an ideal cell source for tissue regeneration. One of the difficulties to overcome before these cells can be used for implantation is to eliminate the use of animal derived components in IPSC cultures. Fetal bovine serum as well as a protein coating is needed for successful IPSC cultures in which the cells proliferate while maintaining their pluripotency. In the search for a xeno-free cell culture system, we identified a specific surface topography on which IPSCs maintained their stemness markers for a prolonged period of time while continuing to proliferate. Obviously, implementing this surface topography as a standard in cell culture plastic for IPSC cultures could greatly reduce the difficulties in translating *in vitro* experiments to clinical application. Unfortunately, it was not possible to create value by valorization of this knowledge due to a conflict in novelty. Analysis of our findings as claimed in our manuscripts gives us: the identification of a surface structure which maintained the undifferentiated state of stem cells without the use of a feeder-layer. Prior to our observations, a Danish group published a paper with a similar scientific message: the identification of a distinct surface structure for undifferentiated expansion of stem cells[7]. Even though the details of the papers

differed greatly (e.g. human IPSCs vs. mouse embryonic stem cells, polystyrene substrates vs. coated silicon, and different defined surface structures) the novelty of our findings was affected. As a result, there was no ground to claim a novelty which could be patented and used for commercialization.

One can imagine that the TopoChip platform is an ideal starting point to explore many more biological models in which surface topography can improve cell culture. To improve our visibility as a platform in the field, we could perform more TopoChip or TopoWellPlate screens in order to identify ideal topographies for, and subsequently distribute TopoWellPlates to interested research groups. In terms of cell-material research, an additional benefit will be the extra amount of data produced as a result of using our defined surface topographies. This growing dataset on topography induced molecular processes can become valuable for compiling the canonical pathway of mechanotransduction (introduced in chapter 1), one of cBITE's main research topics.

The TopoWellPlate as a tissue culture plastic product

Regular 96-well plates cost around 2 euro per plate. However, in order to create an optimized cell culture environment there is also a need for defined culture medium, coatings, gels or a feeder layer, which makes the cell culture much more expensive. Regular tissue culture plates are typically produced using injection moulding. Here, liquid polystyrene (or other polymers) is poured into a mould, creating a multi-well plate once cooled down. Production of TopoWellPlates can be executed using a similar process, and requires in principle only an adjustment of the mould. Here, the cell culture surface area of the mould should be enhanced with the surface topography of interest. Obviously, collaborating with a tissue culture plastic producing company, such as Corning, Nunc, or Greiner, would greatly improve the know-how needed for translating the current more prototype-like state of the TopoWellPlate into a product of industrial quality. Such a collaboration would upgrade the TopoWellPlate as a product from its current TRL3 status towards TRL7/8 which is then ready for TRL9 implementation.

Creating topography enhanced moulds for injection moulding will have an initial cost prize, and per produced TopoWellPlate, a small additional volume of polystyrene to create the topography structure. However, once the production line is created for TopoWellPlates – containing e.g. a surface topography specifically defined for 1 cell type – the production price per TopoWellPlate will approach a traditional well plate production price. And as mentioned above, it will be relatively easy to perform a TopoChip screen to find a surface topography which can be implemented in new biological models. Using this strategy, we will be able to develop optimized TopoWellPlates for any research line.

Introducing a variety of cell type-specific well plates to the market will be revolutionary for *in vitro* cell biology[8]. Since standard cell culturing has already been well-established for decennia – e.g. using regular unpatterned tissue culture plastics – it will take some effort to convince researchers to implement the TopoWellPlate into their protocols. To achieve this, it will be necessary to show the beneficial properties of surface topographies in significant scientific journals, and building a portfolio of topography induced phenotypes. Furthermore, we need to create a network of collaborators in different fields of research which we provide with topographically enhanced cell culture plastics. This will allow them to familiarize themselves with the TopoWellPlate system, and subsequently, share results and experiences among peers. Since the TopoWellPlate is not the first innovation for cell cultureware which aims to become an integral part of the cell cultureware market, we can use previous success stories, such as the one described below, to design a potential market strategy for the TopoWellPlate as well.

In late 2005, a research group led by Sally Meiners published a paper on synthetic electrospun fibers which enhanced neural growth *in vitro*[9]. In this work, the authors emphasized the need for a three-dimensional microenvironment for neural cells *in vitro* to facilitate cell adhesion and neural outgrowth. Their electrospun nanofibers were shown to create such an environment, and furthermore, the synthetic nature of the material and lack of animal derived components made it applicable in a clinical setting. Within a year a second paper was published using this material, in which the authors demonstrated the beneficial effect of the nanofibers for self-renewal of mouse embryonic stem cells[10]. To commercialize this scientific finding and create a cell culture product of industrial quality, Donaldson Co., Surmodics, Inc., and Corning Inc. collaborated in the product development in 2006. Next, in 2008, an application note was released by Corning Inc. on a novel cell culture system – Ultra-Web – in which the benefits of the electrospun nanofibers over collagen coatings was described for hepatocytes[11]. Corning inc. is a leading company in the production of cell cultureware with yearly total expenses for all research and development of approximately 750 million euros. In 2016, Corning's revenues from external costumers for cell culture products were 327 million US dollars[12]. Currently, Ultra-Web multi-well plates are commercially available for € 31.50 per plate (Corning® 96 Well Flat Clear Bottom Black Polystyrene Ultra-Web™ Synthetic Surface Microplates, with Lid, Sterile (Product #3872XX1)) in the corning catalogue. Unfortunately, no detailed information is available about the percentage of revenues for Corning Inc. that is coming from the sales of Ultra-Web cell culture plates.

Once the TopoWellPlate is developed into a cell culture plate of industrial quality, it can probably enter the market for a similar price per plate as the Ultra-Web plates, e.g. € 30.00. Since we estimated the production costs of our plates to be only slightly higher than regular 96-well plates, the profit per well plate could be around 30 – 2 = € 28.00 per plate. A PubMed query revealed that over the last 5 years 2133 papers were published related to primary hepatocytes and drugs. For each of these papers, it can be expected that cells were maintained in culture for multiple weeks during which the experiments were executed. If we assume that

the total cell culture time is 2 months, in which 3 plates are used per week, a publication would typically require 8 × 3 = 24 plates. Following this hypothesis, the profit of the TopoWellPlate could have reached 24 (TopoWellPlates) × 2133 (published papers) × 28 (€ profit per plate) = € 1,433,376 over the last 5 years. This is solely based on published results from academic institutions using primary hepatocytes for drug development or the unravelling of molecular mechanisms. Obviously, similar figures could be applied for other cell types and research lines, and the use of TopoWellPlates in the pharmaceutical industry should give rise to even bigger numbers due to their high experimental capacity.

Conclusion

The TopoWellPlate system has a great potential for economical valorization. It is a cell culture tool which can be potentially implemented in the majority of cell biology laboratories around the world. We can offer those laboratories optimized cell culture plates which can overcome many types of difficulties that researchers are faced with when culturing all these different cell types. Furthermore, as a positive side-effect, all the data generated on the TopoChip-derived defined surface topographies can be used to create a fitting canonical pathway of mechanotransduction.

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